UT Southwestern Internal Medicine Grand Rounds

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Complex Dyslipidemias: Challenges in Management

This is to acknowledge that Zahid Ahmad, M.D. has disclosed that he does not have any financial interests or other relationships with commercial concerns related directly or indirectly to this program. Dr. Ahmad will not be discussing off-label uses in his presentation.

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Particular interests: Disorders of Lipid and Lipoprotein Metabolism

Purpose: To review the challenges in management of patients with complex lipid disorders.

Overview: The past 60 years of lipid lowering drug development has led to multiple medications that are well characterized including statins, niacin, bile acid sequestrants, fibrates, fish oil, and ezetimibe. Still, several challenges remain. A significant amount of individuals have adverse reactions to existing drugs, with the most common adverse reaction being statin induced myopathy. Statin induced myopathy complicates 5-10% of statin users, and no effective approach to treating such patients has been established. High-risk populations (nephrotic syndrome, heterozygous familial hypercholesterolemia) are unable to get to goal on existing drugs, and some individuals (homozygous familial hypercholesterolemia, familial chylomicronemia syndrome) do not respond to any existing drugs. Because LDL-C is a well-established target to improve mortality, a need exists for further options for individuals with statin intolerance and familial hypercholesterolemia. Because severe hypertriglyceridemia leads to recurrent acute pancreatitis, a need exists for TG lowering agents in individuals with familial chylomicronemia syndrome.

Educational Objectives:

1. Understand the scope of statin myopathy
2. Understand familial hypercholesterolemia and treatment challenges
3. Understand familial chylomicronemia syndrome and treatment challenges

I. Introduction

Currently, six classes of lipid lowering agents are available: statins, bile-acid sequestrants, niacin, cholesterol absorption inhibitors, fibrates, and fish oil. As a whole, these lipid lowering drugs are the second most commonly prescribed medication class in the US (1), with statins being the most commonly prescribed agent within this class. The widespread use of statins can be attributed to overwhelming evidence of benefit and safety as well as awareness campaigns directed at physicians and directly to consumers.

These six classes of lipid lowering agents are effective at treating the vast majority of dyslipidemias. However, a few challenges remain. First, approximately 5-10% of statin users develop intolerance. Second, some individuals, such as those with heterozygous familial hypercholesterolemia or nephrotic syndrome, are unable to attain their low-density-lipoprotein cholesterol (LDL-C) goals. Third, there are some rare lipid disorders, such as homozygous familial hypercholesterolemia and familial chylomicronemia syndrome, that respond minimally or not at all to the current lipid lowering agents. I will review a few of the important challenges today.

II. Statin Intolerance

Certainly, statins are powerful, predictable, and consistent LDL-C reducers as well as being over-all well tolerated and having good patient adherence (2). As statin use has become widespread, there is an increased awareness of individuals developing adverse reactions to statins, with the most common adverse reaction being myopathy. Unfortunately, the pathophysiology, and even the terminology, of statin related muscle injury is not yet well established (3) and according to patients, physicians more commonly dismiss rather than acknowledge the possibility of a statin link to muscular symptoms (4). Furthermore, the diagnosis of myopathy is difficult, especially since some of these individuals can have normal serum creatine kinase (CK) levels despite demonstrable weakness and muscle biopsy proven statin-induced myopathy (5).

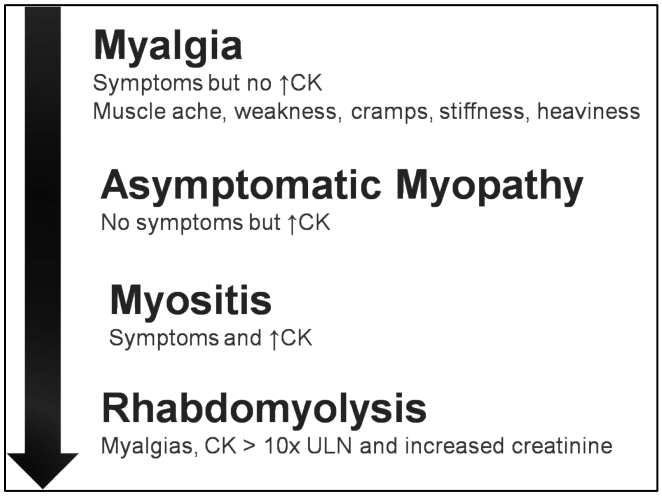


Figure 1: Terms used to define statin myopathy

A. Definitions

There is no well-accepted standard definition of statin induced myopathy. Rather, there are several terms that are typically used to qualify a patient's presentation. The following definitions are adapted from the National Lipid Association (NLA) and American College of Cardiology/American Heart Association (ACC/AHA) recommendations (6, 7) (Figure 1). **Myalgia** is defined as having myopathy symptoms but no elevation in CK. The symptoms can include muscle aches, weakness, cramps, stillness, or "heaviness" while taking a statin. **Asymptomatic myopathy** is defined as having no symptoms but an elevated CK. The CK elevation resolves after the statin is stopped. **Myositis** is defined as having myalgias but with increased CK. **Rhabdomyolysis** occurs when there is breakdown of muscle fibers that leads to the release of muscle fiber contents (myoglobin)

into the bloodstream. It is typically diagnosed by demonstrating increased CK with acute kidney injury and myoglobin in the urine. Although there is no absolute cut-off value for CK elevation, statin induced rhabdomyolysis typically involves CK elevation 10 fold higher than the upper limit of normal.

B. Historical context

The first case of statin-induced myositis was recognized soon after the discovery of the first statin by Drs. Akira Endo and Masao Kuroda in Japan. Dr. Endo and his colleagues documented the case of a 17-year-old girl with homozygous familial hypercholesterolemia who was initially administered a very high dose of mevastatin (8, 9). They then noted "a side effect (muscular weakness at the proximal parts of the extremities with a rise of serum creatine phosphokinase, glutamate-pyruvate transaminase and glutamate-oxaloacetate transaminase activity)." They also noted "these side effects completely disappeared within two weeks after withdrawal of the drug." With lower doses of the mevastatin, "the side effects were no longer experienced."

In 1988, the first cases of rhabdomyolysis were reported as "correspondence" the *New England Journal of Medicine*. In two separate reports, a total of five patients who had undergone cardiac transplantation were described as developing rhabomyolysis after getting lovastatin (10, 11).

C. Characteristics

Although the presentation of statin myopathy varies greatly, there are several characteristic findings. Myalgias involve large, proximal muscle groups and are typically symmetrical. The onset of myalgias occurs within six months of drug initiation, but has been reported to occur at any time during the course of statin administration (12). Statin related muscle complaints improve promptly after drug termination, although it can take up to three months to resolve completely (12).

Occasionally, muscle symptoms or elevated CK do not improve after drug discontinuation. In such cases other myopathies should be investigated (such as polymositis, polymalgia rheumatica, etc). Occasionally, previously asymptomatic inherited myopathies have manifested only after statin administration. Examples of such genetic disorders include heterozygous myophosphorylase deficiency (MPD), homozygous MPD (McArdle disease), heterozygous carnitine palmitoyl-2 (CPT-2) deficiency, and Pompe's disease (13).

Anecdotally, lipidologists have observed that statin myopathy tends to reoccur with other statins, often time more promptly. This suggests a "legacy" or "dose accumulation" effect with statins.

D. Risk Factors

Numerous risk factors for statin induced myopathy have been elucidated from clinical trial experience. These include advanced age, female sex, small body frame and frailty, multisystem disease, high doses of statins, hypothyroidism, alcoholism, grapefruit juice consumption, major surgery or perioperative period, excessive physical activity, history of myopathy while receiving another lipid-lowering therapy, history of CK elevation, unexplained cramps, family history of myopathy, and family history of myopathy while receiving lipid-lowering therapy (3). Ethnicity/race can also be a factor. Asians are known to have a decreased clearance of rosuvastatin (14). Interestingly, African-Americans are known to have higher baseline CK but no increased risk of myopathy (14). Additional treatment related risk factors include high dose statin therapy and interaction with concomitant drugs (fibrates, cyclosporine, antifungals, macrolide antibiotics, HIV-1 protease inhibitors, nefazodone, amiodarone, and verapamil) (3).

E. Frequency

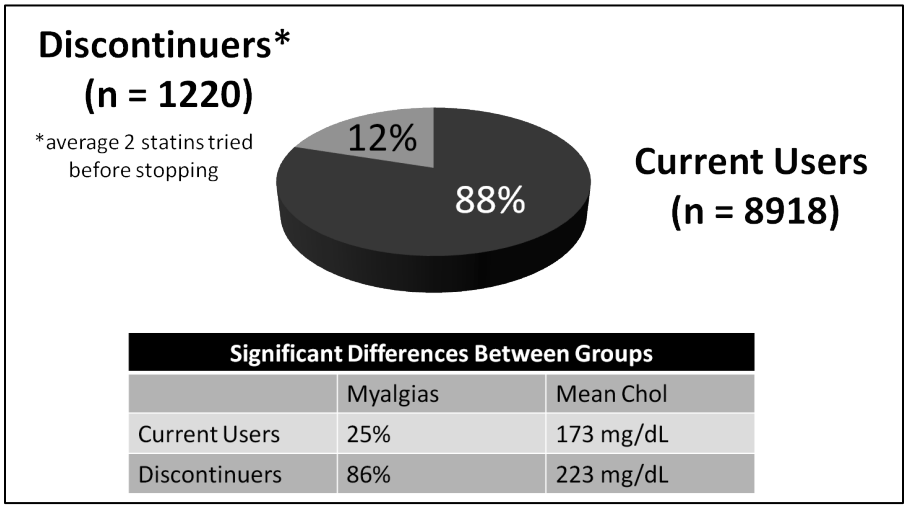
The incidence of statin myopathy in clinical trials is not too impressive (3), Myalgia incidence has been reported to be as little as 2% in clinical trial participants. Myositis and rhabdomyolysis incidences are consistently reported to be as low as 0.05%, and 0.002%, respectively. In fact, if cerivastatin (the only statin to be removed from the market) studies are excluded, the clinical trial frequency of statin induced myopathy is no different than placebo (15).

Table 1: Frequency of muscle symptoms in 7924 adult French patients observed in 2752 primary care practices (PRIMO study) (16)

Statin	Dose	% with muscle symptoms
Simvastatin	40 to 80 mg	18.2%
Atorvastatin	40 to 80 mg	14.9%
Pravastatin	40 mg	10.9%
Fluvastatin XL	80 mg	5.1%

There are several caveats with interpreting safety data from clinical trials of statins. Individuals with a prior history of muscle related symptoms are usually excluded from participation in clinical trials. Most statin trials have included a run-in phase, and the presence of muscle symptoms or increases in CK during this phase excludes these subjects from retention. Finally, many clinical trials have eligibility criteria that exclude individuals with risk factors for myopathy. The recent Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER) study is an example of a study that found no significant myalgias but had broad exclusion criteria. The criteria included previous treatment with any lipid lowering therapy, hepatic dysfunction, elevated CK, increased creatinine, hypothyroidism, alcohol use, drug abuse, inflammatory conditions, and any "medical condition that might compromise safety or successful completion of the study (17)."

Muscle complaints are much more common in clinical practice than reported in clinical trials. Studies such as Prediction of Muscular Risk in Observational conditions (PRIMO, Table 1) (16), an observational survey of muscular side effects in patients receiving high-dosage statin treatment in general practice clinics in France, give an idea of how often these symptoms occur. Overall, muscular symptoms were reported by 10.5% of statin users, with a median time of onset of one month following initiation of statin therapy. The highest prevalence was 18.2% amongst users of simvastatin 40-80 mg/day.



Significant Differences Between Groups		
	Myalgias	Mean Chol
Current Users	25%	173 mg/dL
Discontinuers	86%	223 mg/dL

Figure 2: Results of the USAGE survey of statin use in the US (18).

More recently, the USAGE study (http://www.statinusage.com) surveyed > 10,000 individuals in the US who were prescribed statins and found that 12% had discontinued their statin. 86% of those who discontinued statins experienced muscle pain or weakness, compared to 25% among current users (18) (Figure 2). Discontinuers were more likely to have multiple co-morbid conditions, and on average, two statins were tried before stopping.

Table 2: Results of the STOMP study, **p = 0.05

	Atorvastatin 80 mg daily (n = 203)	Placebo (n = 207)
Myalgia** (%)	9%	5%
Muscle group(s)	Legs	Generalized

The recent Effect of Statin Medications on Muscle Performance (STOMP) study was a randomized double blind clinical trial designed to examine the effects of statins on muscular complaints (19). The definition of myalgias required resolution of muscle symptoms promptly after stopping study medication and reappearance of symptoms on restarting the medication. Using this strict definition, 9% of statin users developed myalgias.

According to the CDC data from 2003, 37% of the US population has at least two risk factors for heart disease, making them potential statin users. If 5-10% of these individuals experience statin-induced muscular symptoms, then the estimated prevalence of statin myopathy is 5 -10 million in the US (20).

F. Is Statin Myopathy Real?

According to a study published in 2007, physicians more commonly dismiss rather than acknowledge the possibility of a statin link to muscular symptoms (4). Several prominent lipidologists continue to insist that statin myopathy is a “disease of the mind.” However, there are several lines of evidence that suggest that statins have a toxic effect on muscles.

In the 1990’s, Thompson et al showed that patients treated with lovastatin had mildly increased CK levels after downhill walking (21). More recently, the STOMP study assessed symptoms and measured CK, exercise capacity, and muscle strength before and after atorvastatin 80 mg or placebo was administered for 6 months to 420 healthy, statin-naïve subjects (19). No differences were found in exercise capacity or muscle strength, but they observed a mild increase in CK among atorvastatin users, suggesting that statins produce a mild muscle injury even among asymptomatic subjects.

The strongest evidence for the existence of statin induced myopathy comes from muscle biopsy studies. Skeletal muscle biopsies in patients with statin induced myopathy have demonstrated necrotizing myopathy (22-24). Alarmingly, a muscle biopsy study of asymptomatic statin users showed evidence of skeletal muscle damage when compared to controls, suggesting that statins may also be causing myocyte damage at a subclinical level (25).

G. Pathopharmacology and Pharmacogenetics

The exact pathology at the level of skeletal muscle remains unclear. Statins inhibit an early step of the cholesterol biosynthesis pathway, and it is possible that intermediates in the pathway play a role in statin myopathy. It is also well known that statin myopathy is directly related to exposure. As such, several drug-drug interactions have been described that involve phase 1 and phase 2 metabolism of statins. It has also become clear that drug transporters are involved, and recent genetic studies have implicated variations in drug transporter genes among patients with statin intolerance.

Statins competitively inhibit the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which catalyses the third and rate-limiting step of the cholesterol biosynthesis pathway. Isoprenoids are secondarily reduced. This leads to an impairment of multiple pathways, including: (1) selenocysteine (Sec) transfer RNA (tRNA) isopentenylation, (2) dolichol-mediated N-linked glycosylation, (3) protein prenylation by farnesyl-pyrophosphate and geranylgeranyl-pyrophosphate, and (4) coenzyme Q10 (ubiquinone; CoQ10) tail synthesis, which may influence antioxidant and respiratory chain capacities within the cell (26).

Treatment with statins (pravastatin, simvastatin, and atorvastatin) has been shown to reduce CoQ10 plasma levels in several studies (27-29), although one study did not show any reduction in CoQ10 levels in

patients given pravastatin or atorvastatin (30) . CoQ10 supplementation has been studied in three small but well designed, randomized, double blind trials that yielded conflicting results (31, 32). In a small trial (n of approximately 40), Caso et al showed that coenzyme Q10 100 mg/day reduced pain severity in patients with myopathic symptoms when compared to patients taking Vitamin E (31). In another small pilot trial, Young et al randomized 44 patients with statin myopathy to coenzyme Q10 200 mg/day or placebo for 12 weeks (32). There was no difference between the two groups in myalgias scores. In the third and most recently performed study, Bookstaver et al randomized 76 patients with apparent statin induced myalgias to coQ10 60 mg twice daily or placebo (33). All patients continued their statins. At one month, there was no difference in pain scores, and the authors concluded that CoQ10 did not produce a greater response than placebo in the treatment of presumed statin-induced myalgias. Several coenzyme Q10 trials are ongoing and may help answer the question of whether coenzyme Q10 may help relieve or prevent myopathic symptoms. A search of clinicaltrials.gov identifies at least four such studies (clinicaltrials.gov #'s: NCT00997269, NCT01140308, NCT01026311, and NCT00716612).

Another potential mechanism of statin myopathy is Vitamin D deficiency. The immediate precursor to cholesterol is 7-dehydrocholesterol, and Vitamin D is produced when 7-dehydrocholesterol is converted to vitamin D3 in the skin by ultraviolet B light. The vitamin D receptor is present in skeletal muscle, and vitamin D deficiency can cause myopathy. However, statins have been shown to have a neutral effect or even increase levels of Vitamin D (34). A clear relationship has not been established between statins, Vitamin D, and statin myopathy, but a randomized placebo controlled trial is currently underway to examine whether Vitamin D supplementation will reduce statin myopathy (clinicaltrials.gov #: NCT01400009).

The myopathic effect of statins increased with increasing steady state blood levels, which is largely affected by drug metabolism and genetic variants in drug transporters. Drug metabolism is divided into phase 1 and phase 2 reactions. Phase 1 reactions typically introduce a functional group via oxidation or reduction. All statins, except pravastatin, undergo oxidation via the cytochrome P450 (CYP) system (Table 3). Many of the drug-drug interactions that occur with statins involve the CYP3A4 system (35). Atorvastatin, lovastatin, and simvastatin are all metabolized by this system. Interacting drugs (inhibitors/substrates of CYP3A4) include azole antifungals, cimetidine, clarithromycin, erythromycin, cyclosporine, diltiazem, HIV-1 protease inhibitors, and grapefruit juice (35). These inhibitors and substrates can lead to decreased statin metabolism, increased blood concentration of the statin or active metabolite, and increased risk for muscle toxicity. Note that pravastatin is not metabolized by the P450 system, prompting it to often be called the "safest statin." Pitavastatin undergoes only minor metabolism by the P450 system and is mainly metabolized by phase 2 reactions. There is only limited clinical experience with pitavastatin in the US.

Table 3: Cytochrome P450 (CYP) Enzymes Involved in Statin Metabolism (35)

Statin	CYP3A4	CYP2C9	CYP2C8	CYP2C19
Atorvastatin	x			
Fluvastatin		x	x	
Lovastatin	x			
Pitavastatin		x	x	
Pravastatin				
Rosuvastatin		x		x
Simvastastin	x			

Phase 2 reactions involve conjugation reactions with other molecules. All statins undergo glucuronidation by UDP glucuronyltransferases 1A1 and 1A3. Competing drugs include gemfibrozil and cyclosporine, and concomitant administration with statins can results in increased statin exposure of 20% or more. Of note, the concomitant use of gemfibrozil and simvastatin is contraindicated primarily because of this interaction during phase 2 metabolism. The Food and Drug Administration (FDA) recently changed the label for simvastatin to reflect this drug-drug interaction. For the other statins, a general rule of thumb is to use only 10 mg when also administering gemfibrozil. Cyclosporine use contraindicates statin use except for pravastatin and low doses of fluvastatin or rosuvastatin.

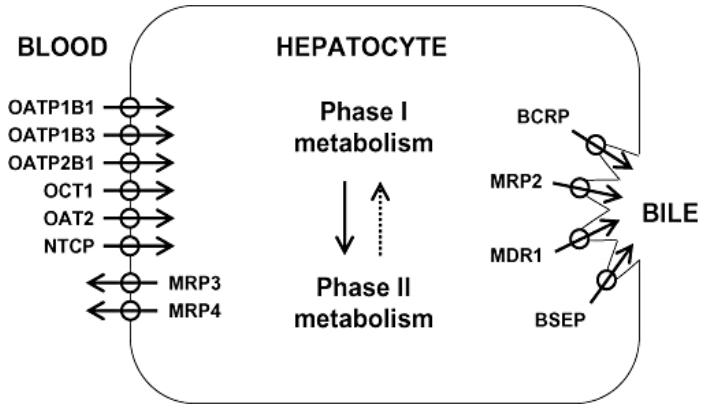


Figure 3: Drug transporters affecting hepatic uptake and excretion of drugs – from (36)

Inhibition of hepatic uptake transporters by co-medications and reduced transport activity due to genetic polymorphisms also have the potential to increase systemic (and thereby muscle) exposure (37). The most studied drug transporter implicated in statin myopathy is organic anion transporting polypeptide 1B1 (OATP1B1). OATP1B1 is expressed in liver hepatocytes on the basal-lateral side of the sinusoidal membrane, and it serves to bring substrates into the liver from the blood stream. OATP1B1 can thus mediate the hepatic elimination of these compounds. Inhibitors and substrates of OATP1B1 include all of the currently available statins, cerivastatin, gemfibrozil, cyclosporine, and many other pharmacologic agents.

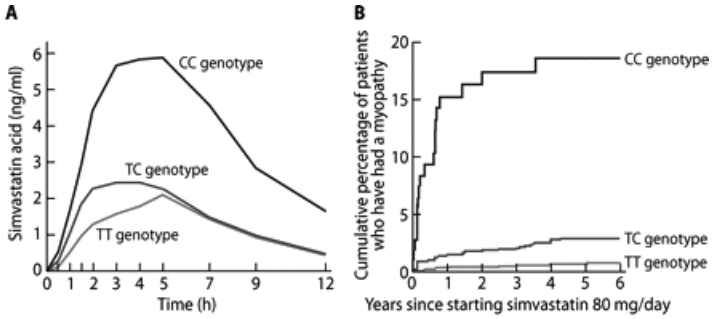


Figure 4: Effects of SLCO1B1 c.521T>C variant on (A) plasma concentrations of active simvastatin acid after a single 40-mg dose of simvastatin in healthy volunteers, and (B) on the cumulative incidence of myopathy during treatment with 80 mg/day simvastatin daily (38).

OATP1B1 is encoded by the gene *SLCO1B1*. A genome wide association study compared 85 myopathy cases with 90 controls, all of whom were taking 80 mg of simvastatin daily (38). The scan yielded a single strong association of myopathy with the rs4363657 (c.521T>C) single-nucleotide polymorphism (SNP) located within *SLCO1B1.* More than 60% of the myopathy cases studies were attributed to the C allele. The odds ratio for myopathy was 4.3 for one C allele and 17.4 for both C alleles (Figure 3). After a single 40 mg dose of simvastatin in healthy volunteers, simvastatin acid levels were almost three fold higher in those with both C alleles when compared to those with both T alleles (37, 38). Subsequently, *SLCO1B1* genotype has been found to influence the plasma concentration of all statins (39). The most dramatic effect is seen with simvastatin acid, which can have 221% increase in exposure (Figure 4). Consequently, it is not surprising that individuals with genetic variations in SLCO1B1 have an increased incidence of rhabodmyolysis during statin therapy (38) .

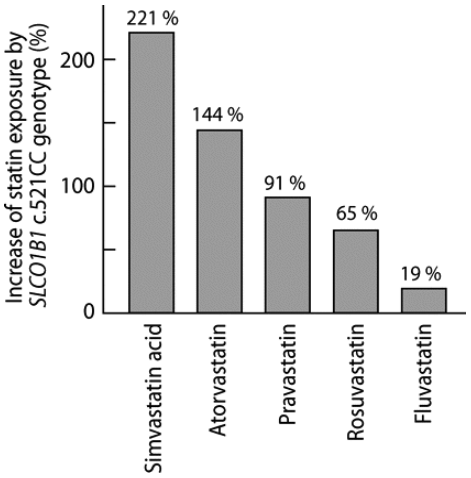


Figure 5: Effects of the *SLCO1B1* c.521T>C variant on the exposure (area under the plasma statin concentration-time curve) to different statins (40).

It would be expected that the *SLCO1B1* c.521T>C SNP would be associated with an attenuated cholesterol-lowering response to statin therapy. In the Heart Protection Study, the low-density lipoprotein cholesterol-lowering effect of 40 mg/day simvastatin was only 1.3% smaller per copy of C allele (37, 38). Thus, the *SLCO1B1* c.521C increases plasma statin concentrations and the risk of myopathy, without a dramatic increase in cholesterol-lowering efficacy.

Although not as well studied, several other hepatic transporters have been implicated in statin myopathy including (but not limited to) breast cancer resistance protein (BRCP), organic anion transporting polypeptide 1BP3 (OAT1BP3), and multidrug resistance protein 1 (MDR1) (37). Variations in the genes encoding these proteins also influence the plasma concentration of statins (40). These transporter genes have now been shown to be expressed in human skeletal muscle, where they may determine local exposure and toxicity of statins (41).

H. Therapeutic Strategies

There is no consensus on management of patients with statin intolerance. At our center, the general approach is the following. First, we reassess the patient with regards to their need for a statin. The benefit of the statin must always be balanced with the risks. Individuals who are low-risk for CHD and have experienced statin myopathy may no longer tip the risk-benefit scale towards benefit. In individuals at moderate or high CHD risk who develop myalgias (but not myositis or rhabdomyolysis), we often try a “statin challenge.” This challenge involves stopping the statin for two weeks to see if the symptoms go away, then restarting the statin to see if the symptoms return. Second, we identify concomitant medications that may be contributing to increased statin exposure. Third, we maximize therapeutic lifestyle changes, including the use of plant stanols. Fourth, if CK is not elevated, we typically try a different statin. This step (and the following steps) involve a great deal of “trial-and-error.” Fifth, if CK is elevated or the patient is not willing to take a statin, we try non-statin lipid lowering agents. Sixth, we try “desperate measures” such as non-traditional statin doses and rarely LDL-apheresis.

Of course, the best management of statin intolerance may be prevention. Prior to prescribing a statin, a “check-list” type of evaluation may be beneficial. Family history of myopathy, secondary causes of muscle problems, and potential drug-drug interactions should be identified. In light of recent findings that statins increase the risk of developing diabetes, diabetes risk factors should be identified. These steps may help identify those who require more careful monitoring or more cautious dosing. At-risk patients can also be informed about the potential side-effects and the need to report them as soon as possible.

I. Future Direction

a. Pharmacogenomics

SLCO1B1 genotyping for rs4149056 (c.521T>C) is currently commercially available (Berkeley HeartLab, Bostonheart Diagnostics), and this genotyping may be clinically meaningful because the *SLCO1B1* c.521T>C SNP and interacting drugs may have additive effects on statin pharmacokinetics. However, the clinical utility of this genotyping has not yet been studied methodically. In addition, other variants in *SLCO1B*1 and variants in other genes have been found to decrease transport activity of statins (37), and the risk of myopathy may be related to multiple variations with small cumulative effects. A search of clinicaltrials.gov reveals several studies related to the study of genetic variations associated with statin induced myopathy (NCT00549029, NCT00767130).

b. Potential New Therapies for Statin Intolerant Patients: PCSK9 Inhibition

Proprotein convertase subtilisin/kexin 9 (PCSK9) is a member of the proprotein convertase family of zymogens and modulates plasma LDL-C levels by promoting degradation of LDL receptors. PCSK9 was identified via linkage analysis in three French families with autosomal dominant hypercholesterolemia but no mutations in *LDLR* or *APOB* (10). These individuals were found to have gain-of function mutations (Figure 6).

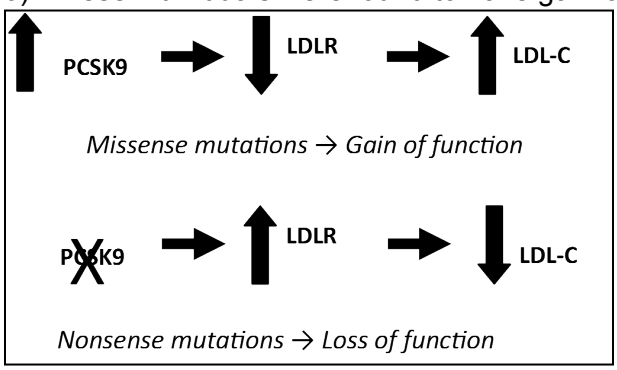


Figure 6: Effect of PCSK9 gain of function vs loss of function mutations

Conversely, Hobbs et al established that loss-of-function mutations in *PCSK9* are associated with hypocholesterolemia and protection from coronary heart disease (CHD) (42, 43). Thus far, PCSK9 deficiency has not been associated with obvious ill effects, making PCSK9 an attractive target for lipid lowering.

The Goal Achievement after Utilizing an anti-PCSK9 antibody in Statin Intolerant Subjects (GAUSS) study assessed the efficacy and safety of a monoclonal antibody to PCSK9 (AMG145, Amgen) in patients (n = 160) with a documented history of muscle-related adverse effects with statins (intolerable myalgias or myositis) (44). Patients could continue low dose statins if they were able to tolerate them. After 12 weeks of treatment, LDL-C reduction was approximately 50% in the group that received only the study drug at the highest dose (other groups also received ezetemibe). This LDL-C reduction is similar to statins and greater than currently available non-statin lipid lowering medications. Among the patients who got both AMG145 and ezetemibe, 90% got to their goal LDL-C. Overall, the drug was well-tolerated, but myalgia did occur in six patients receiving AMG145 only. Two patients in the AMG145 only group developed CK levels greater than 10 times the upper limit of normal. Both cases occurred after exercise. Future studies of longer duration and larger sample size are needed to establish the safety of PCSK9 inhibition as well as the cardiovascular benefits. Nevertheless, PCSK9 inhibition is perhaps the most promising LDL-C lowering therapy for patients with statin intolerance.

III. Familial Hypercholesterolemia

Because of their very high baseline LDL-C values, individuals with disorders such as heterozygous familial hypercholesterolemia and nephrotic syndrome require intensive statin therapy yet still don't get to their LDL-C targets. Compared with the low-dose statin therapy, intensive statin therapy has been associated with increased incidence of discontinuation, hepatotoxicity, and myalgia (45-49). In addition, these individuals often require various combinations of statins, bile acid binding sequestrants, ezetimibe, and niacin. However, there is

lack of documented clinical trial evidence for the efficacy of many of these combination regimens in the prevention of CHD (45).

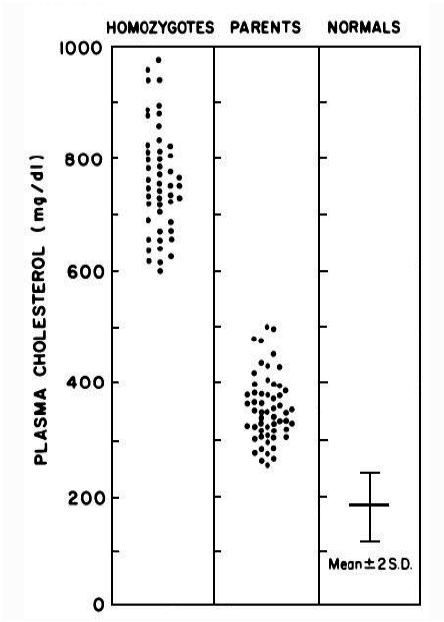


Figure 7: Plasma cholesterol in patients with homozygous FH, their parents (obligate heterozygous FH), and normals (50)

Familial hypercholesterolemia (FH, OMIM #143890) is an autosomal codominant disorder caused by mutations in the LDL receptor gene. (Mutations in apolipoprotein B100 and PCSK9 cause a similar phenotype, but are much rarer). Those individuals who are heterozygous for one of these mutations typically have total cholesterol > 300 mg/dL (Figure 7), premature arcus senilis, tendon xanthomas, and premature CHD. Homozygous patients typically have total cholesterol > 500 mg/dL and develop CHD at very early ages.

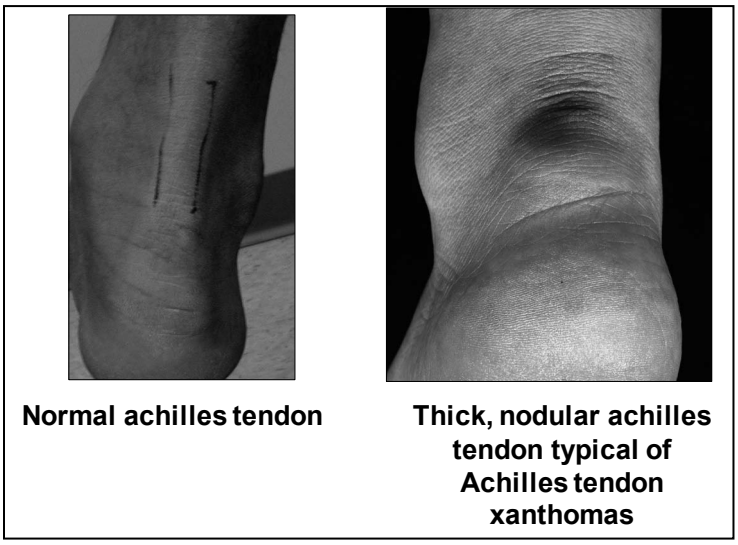


Figure 8: Achilles tendon xanthomas

FH was one of the first genetic disorders of lipid metabolism to be characterized at the molecular level. Brown and Goldstein (at UT Southwestern), in the 1970s, discovered that the disease is caused by mutations in the LDLR, a cellular surface protein that recognizes and internalizes LDL particles (51). Mutations in *LDLR* account for the majority of monogenic hypercholesterolemia cases (52) and more than 1000 unique disease-causing variants have been reported (www.ucl.ac.uk/ldlr/LOVDv.1.1.0/)(52). Worldwide, the prevalence of

heterozygous FH due to heterozygous *LDLR* variants is estimated to be 1 in 500 (53), making it the most frequent Mendelian disorder. In fact among survivors of myocardial infarcts, the frequency of heterozygotes is about 1:20. Homozygous individuals, although rare, have a much more severe phenotype.

A. Homozygous FH Patients Don't Respond Well to Lipid Lowering Agents

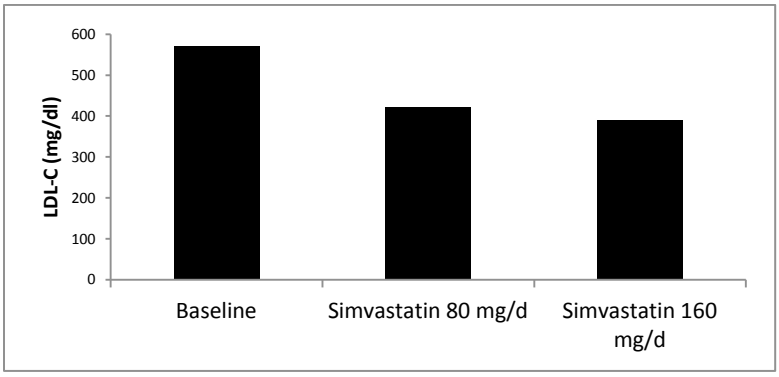


Figure 9: Response to high dose simvastatin in patients with homozygous FH (54).

Homozygous familial hypercholesterolemia, although rare, results in vascular disease as early as the first or second generations of life. Unfortunately, these individuals respond minimally to existing lipid lowering agents, especially in the case of homozygous null *LDLR* mutations. A trial of simvastatin in 8 homozygous FH patients examined the effects of very high simvastatin doses (160 mg/day) on LDL-C (Figure 9). LDL-C reduced by 30%, but levels were still in the 300-400's mg/dL range (54). Thus, even amongst those homozygous FH who have some response to lipid lowering agents, they are not able to get to goals. Often the only therapeutic option is LDL-apheresis (see below).

B. Heterozygous FH Patients are Unable to Achieve LDL-C Goals.

Treatment options for heterozygous FH include therapeutic lifestyle changes, lipid lowering agents, and LDL-apheresis. Therapeutic lifestyle changes have minimal effects on lipids in FH patients, primarily because their LDL-C level is heavily influenced by genetics rather than environment. Lipid lowering agents include statins, bile-acid binding resins, and niacin. If needed, LDL-apheresis is an option for heterozygous FH patients who cannot lower their LDL-C sufficiently with lipid lowering agents.

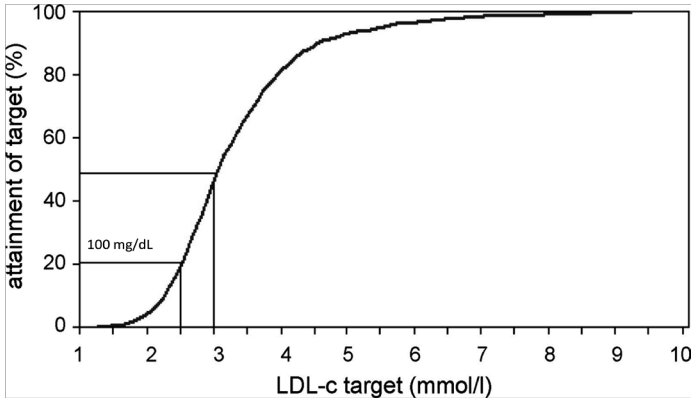


Figure 10: Attainment of LDL-C target in patients with heterozygous LDLR mutations (N=1249, Netherlands) (55)

In the Netherlands, where heterozygous FH is relatively common (1:400), a molecular screening program was set up to identify FH patients. A recent survey of their data revealed that only 22% of treated individuals with FH achieved their LDL goal after 2 years despite an average of 44% reduction in LDL-C (Figure 10) (55) . Even with the most potent treatment regimens, the authors estimate that the Dutch target level could not be reached in many cases. Similarly, in Norway, 6 months after getting a genetic diagnosis of FH and being started on lipid lowering medications, serum LDL-C was reduced by only 25% and a total of only

15.5% of FH patients had total cholesterol at goal (56). In a UK audit of 733 FH patient records, over 89% of patients were treated with statins, yet only 33% achieved target (57). Thus there is a need for additional therapeutic options to lower LDL-C in patients with FH.

C. LDL-Apheresis

LDL-apheresis is a form of apheresis, resembling dialysis, which eliminates LDL-C from the bloodstream (See Figure 11 for an example). LDL-apheresis works by leading venous blood through a column coated with antibodies to apolipoprotein B (the main protein of LDL particles), dextran sulfate or polyacrylate, or by precipitating LDL-C with heparin at low pH. Rarely, LDL-apheresis is complicated by post-procedural bleeding, hypotension, or allergic reactions.

LDL-apheresis has been in use since 1975 for homozygous FH (58), and has been shown to improve survival (59). Two randomized trials studied LDL-apheresis for heterozygous FH patients, but did not find any significant differences in the primary endpoint, quantitative coronary angiography, after two years (60, 61). Thus, it is thought that LDL-apheresis offers no advantage in drug-responsive patients.

Since 1996, the FDA has approved three uses for LDL-apheresis. As part of the qualifications, patients must have failed a six-month trial of diet and maximal drug therapy. For children, exceptions are made for early initiation.

1. Homozygous patients with LDL-C levels greater than 500 mg/dL
2. Heterozygous patients with LDL-C greater than 200 mg/dL with a documented history of CHD
3. Heterozygous patients with LDL-C greater than 300 mg/dL without CHD.

There are two major issues (in addition to being an invasive procedure) to the use of LDL-apheresis. First is cost. At our center, each apheresis treatment costs $ 4000. Homozygous patients typically require one treatment per week, making the yearly cost over $ 200,000. The yearly cost of LDL-apheresis is similar to the most expensive drugs in the world (http://www.forbes.com/2010/02/19/expensive-drugs-cost-business-healthcare-rare-diseases.html) and is much higher than hemodialysis for end-stage-renal disease ($ 70,000/year). There is a need for additional lipid-lowering agents to reduce the cost burden of LDL-apheresis. Second, there is low availability of centers that can provide LDL-apheresis procedures. Patients in rural areas, for example, have a difficult time finding a center for weekly procedure. For these patients, there is a therapeutic need for efficacious treatment modalities that can be distributed through traditional routes.

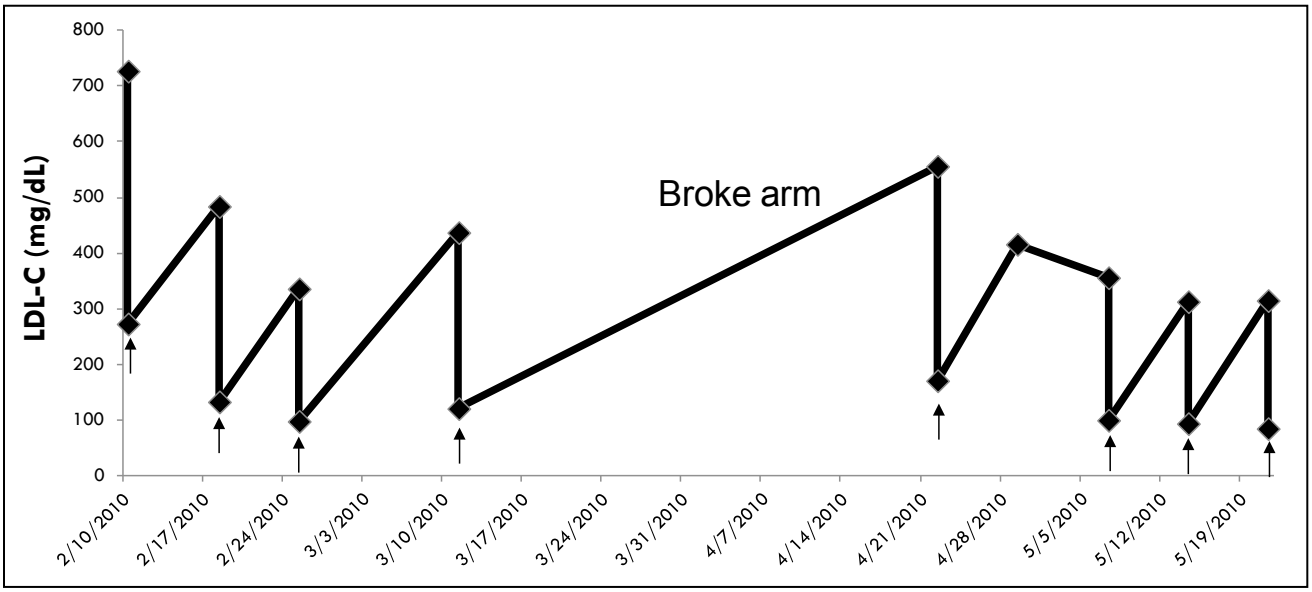


Figure 11: Example of LDL-apheresis effects on LDL-C. Data is from a 23 year old female patient with homozygous FH who attends the Parkland Lipid Clinic. Arrows represent LDL-C measurements immediately after LDL-apheresis.

D. Potential New Therapeutic Options for FH

a. PCSK9 Inhibition

Two monoclonal antibodies to PCSK9 have been studied in patients with heterozygous FH. Regeneron Pharmaceuticals product, REGN727, was administered to patients with heterozygous FH as part of a larger study (62) and in a dedicated separate study (63). In both studies, REGN727 was well-tolerated and LDL-C was reduced by 40-50%. In the Reducton of LDL-C with PCSK9 Inhibition in Heterozygous Familial Hypercholesterolemia Disorder (RUTHERFORD) study , Amgen's product,AMG145, reduced LDL-C up to 55% and 89% of patients on the highest dose reached a goal LDL-C of 100 mg/dl (64). There was no difference in muscle related adverse events when compared to placebo, but a few subjects on the study drug developed asymptomatic elevations in CK related to strenuous exercise. Otherwise, there were no notable differences in the safety profile relative to placebo. Based on these studies, PCSK9 inhibition may offer a new effective treatment option to further reduce LDL-C in heterozygous FH patients unable to achieve optimal LDL-C targets on current medications.

b. Apolipoprotein B (ApoB) Antisense Inhibitors

ApoB is an essential structural and receptor-binding component of all atherogenic lipoproteins, including LDL and its metabolic precursors, intermediate-density lipoprotein (IDL) and very-low density lipoprotein (VLDL). The Apo-B gene is expressed in both hepatocytes and intestinal epithelial cells. However, in liver cells, its product is a 500 kD protein called Apo-B100 whereas in intestine cells, because of RNA editing by Apobec1, its product is a smaller protein called Apo-B48.

Inhibition of ApoB production in hepatocytes is an attractive novel target that would decrease serum cholesterol without involving the LDL-receptor, as statins and bile acid sequestrants do. As such, ApoB would be a therapeutic target in those individuals with mutations in *LDLR*. One promising such drug is Mipomersen (Isis Pharmaceuticals), which is an antisense oligonucleotide. Such drugs are short sequences of nucleotides, and in the case of Mipomersen, it is complimentary to a 20 nucleotide segment of the coding region for the mRNA for Apo B100. Binding of the drug to mRNA results in the mRNA being targeted for degradation by Rnase H rather than translation to the ApoB protein product.

A phase III clinical trial of Mipomersen 200 mg SQ weekly in 51 patients with homozygous familial hypercholesterolemia demonstrated a 25% reduction versus 3% for placebo in levels of LDL-C after 26 weeks of treatment (65). One question that arises is whether inhibition of VLDL production leads to hepatic triglyceride accumulation since hepatocytes are the site of *de novo* TG synthesis that is usually packaged into VLDL. In fact, a few patients (7%) who were administered Mipomersen had a rise in ALT > 3x ULN (presented as abstract Drugs Affecting Lipid Metabolism 2007). Other safety issues include injection site reactions and flu-like symptoms, and of course, the cardiovascular benefit is not established.

c. Microsomal Triglyceride Transfer (MTP) Protein Inhibitors

Microsomal triglyceride transfer protein (MTP) assists in the initial packaging of cholesterol esters and triglycerides into both chylomicrons and VLDL in intestinal cells and hepatocytes, respectively. Two systemic MTP inhibitors were developed in the early 2000's. *Bayer* developed Implitapide, but found it to cause excessive transaminitis in phase II trials and development was discontinued in 2001. *Bristol-Meyers Squibb* developed BMS-201038 but phase 1 trials (2003) had high rate of hepatic steatosis. The drug found its way to individual academic investigators including Dr. Daniel Rader and his colleagues at U Penn. They administered the drug to six homozygous FH patients in an open label dose escalation study to evaluate safety and efficacy (66). After four weeks of therapy, TG decreased by 65% and LDL-C decreased by 51% (1 mg dose). However, ALT and hepatic fat rose in four out six patients.

Despite these setbacks, Aegerion Pharmaceuticals continued to pursue approval from the FDA for homozygous FH with the rationale that homozygous FH is a severe disease with limited treatment options. Thus, the risk-to-benefit profile is favorable. Aegerion was successful as lomitapide was FDA approved for use in homozygous FH on December 24, 2012. The yearly cost for lomitapide is estimated to be $200,000 –

300,000, similar to the cost of LDL-apheresis. The major benefit for lomitapide would be for patients who cannot get LDL-apheresis, especially since lomitapide is dosed orally. The cardiovascular benefit is yet to be established, and liver fat accumulation remains a concern that may limit lomitapide's more generalized use in patients with heterozygous FH.

The FDA based its approval on a 78 week Phase III study that evaluated the safety and effectiveness of the medicine to reduce LDL-C levels in 29 adult patients with homozygous FH (67). When added to the existing lipid-lowering therapy, lomitapide significantly reduced LDL-C from a baseline average of 336 mg/dL to 190 mg/dL (40% reduction) at 26 weeks (efficacy phase of study). Ten of the 29 patients in the study had at least one elevation in liver enzymes greater than or equal to three times the upper limit of normal. Four of those patients experienced liver enzymes greater than or equal to five times the upper limit of normal. Hepatic fat increased from a baseline of one percent to a median absolute increase of six percent at 26 and 78 weeks.

IV. Familial Chylomicronemia Syndrome

According to data from the National Health and Nutrition Examination Survey (NHANES), 2% of the population has triglycerides above 500 mg/dL. The majority of these individuals have secondary causes (i.e. uncontrolled diabetes, alcoholism, kidney disease, lipodystrophy, or drugs such as estrogen) that can be readily identified. Conversely, a small subset of these individuals is born with rare genetic diseases that result in lifelong severe triglyceride elevations despite the absence of any secondary factors. Such individuals have familial chylomicronemia syndrome (Type 1 hyperlipoproteinemia). Familial chylomicronemia syndrome (OMIM# 238600) is a rare autosomal recessive disorder that is characterized by massive hypertriglyceridemia (typically above 2000 mg/dL) and its complications. The pathophysiology is related to a defect in removal of chylomicrons and other triglyceride-rich lipoproteins.

A. Genetics

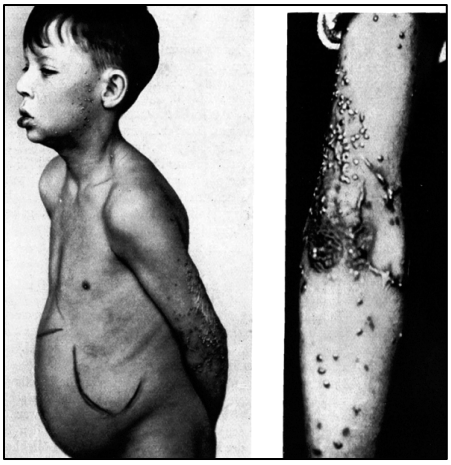


Figure 12: First case of familial chylomicronemia syndrome. Left: young boy with multiple abdominal scars (from surgery for abdominal pain) and protuberant abdomen (from hepatomegaly). Right: eruptive xanthomas

Burger and Griitz first described the clinical syndrome in 1932 in a young male offspring of a consanguineous marriage (Figure 12). He was a young boy with extensive eruptive xanthomas, hepatosplenomegaly, and milky plasma, and he had undergone multiple surgeries looking for the etiology of abdominal pain. After initiation of a low fat diet, many of his complications improved greatly.

In 1989, the first of many lipoprotein lipase (LPL) structural gene defects was described and since then roughly 60 mutations have been described (68). In the circulation, the enzyme LPL catalyzes the release of free fatty acids from chylomicron and VLDL triglycerides. Thus a deficiency in LPL (or in the enzymes and proteins associated with it) results in accumulation of circulating chylomicrons and VLDL. Several additional genetic defects have been found to cause familial chylomicronemia syndrome, including apolipoprotein C2 (*APOC2*) (69), and more recently, apolipoprotein A5 (*APOA5*) (70, 71), lipase maturation factor 1 (*LMF1*) (72) and glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (*GPIHBP1*) (73).

B. Characteristics

The massive triglyceride elevations result in several classic findings. Fasting plasma typically turns milky, referred to as lipemia. Several physical exam findings are characteristic. Lipemia retinalis is a pale pink appearance of retinal blood vessels due to light scattering of large chylomicron particles. Hepatosplenomegaly occurs due to accumulation of triglycerides in the liver and spleen. Triglycerides can also accumulate in the skin, resulting in eruptive (cutaneous) xanthomas. These appear as crops of discrete yellow papules on an erythematous base on the back, buttocks, and extensor aspects of elbows and knees. The lesions will clear when TG return to normal.

The most severe manifestation of chylomicronemia is pancreatitis, which can be lethal. In the general population, hypertriglyceridemia is the third most common cause of pancreatitis. The exact pathophysiology of hypertriglyceridemia induced pancreatitis remains unknown; however patients with familial chylomicronemia syndrome develop pancreatitis frequently. The odds ratio of developing pancreatitis among patients with hypertriglyceridemia due to familial chylomicronemia syndrome is estimated to be approximately 360 (74). The pancreatitis is typically acute, recurrent, and can have normal amylase and lipase levels

C. Current Treatment Options

Currently, no drug therapy for familial chylomicronemia syndrome is available. Patients are managed by an extremely low fat diet: 15% fat in the diet. This diet can typically reduce the triglycerides to less than 2000 mg/dL, greatly reducing the risk of pancreatitis. However, diet therapy has limited durability and is difficult to maintain, especially with the current environment of widely available and cheap calorie-dense processed foods.

D. Treatments currently under investigation

Two promising drugs are currently being investigated for familial chylomicronemia syndrome: inhibitors of diacylglycerol O-acyltransferase 1 (DGAT1) and intestinal specific MTP inhibitors. The final step in TG synthesis is catalyzed by DGAT enzymes. DGAT1 knockout mice are lean, resistant to diet induced obesity, and have increased insulin sensitivity (75). Hence, DGAT1 is considered a potential therapeutic target for treating obesity and related metabolic disorders. By inhibiting TG synthesis, DGAT1 inhibition could potentially reduce circulating triglycerides in patients with hypertriglyceridemia. Recently, Farese and co-workers reported the first human case of DGAT1 deficiency which presented with a congenital diarrheal disorder (76). Oddly, the patients had mild elevations in triglycerides. Regardless, LCQ908, a DGAT1 inhibitor, is currently being evaluated in a randomized, double-blind, placebo controlled study to assess efficacy, safety and tolerability in patients with familial chylomicronemia syndrome (clinicaltrials.gov #: NCT01514461).

The use of systemic MTP inhibitors will likely be limited by hepatic accumulation of triglycerides, which could eventually lead to cirrhosis. This would be especially troubling in patients with familial chylomicronemia syndrome, since these patients already accumulate TGs in their liver. One alternative is targeting MTP in only intestinal cells. Intestinal specific MTP inhibitors may have a better safety profile since they would not affect hepatocytes. One such drug, SLx-4090, has been developed by *Surface Logix.* In early trials (abstracts from Scientific Sessions 2006, Prince et al J Lipidology), SLx-4090 had no effect on liver function and decreased postprandial triglycerides up to 50% in healthy subjects. The only side effects noted were headache, flatulence, and diarrhea. Such a drug could effectively treat chylomicronemia and as such, provide a pharmacological intervention for those individuals with familial chylomicronemia syndrome. We are soon initiating a trial of SLx-4090 in patients with familial chylomicronemia at UT Southwestern.

V. Conclusion

In conclusion, the past 60 years of lipid lowering drug development has led to multiple medications that are well characterized including niacin, bile acid sequestrants, fibrates, statins, fish oil, and ezetimibe. Still, several challenges remain. A significant amount of individuals have adverse reactions to existing drugs, with the most common adverse reaction being statin induced myopathy. Statin induced myopathy complicates 5-10% of statin users, and no effective approach to treating such patients has been established. High-risk

populations (nephrotic syndrome, het FH) are unable to get to goal on existing drugs, and some individuals (hom FH, familial chylomicronemia syndrome) do not respond to any existing drugs. Because LDL-C is a well-established target to improve mortality, a need exists for further options for individuals with statin intolerance and FH. A few promising therapeutic targets to reduce LDL-C include apoB antisense inhibition, MTP inhibition, and PCSK9 inhibition. Because severe hypertriglyceridemia leads to recurrent acute pancreatitis, a need exists for TG lowering agents in individuals with familial chylomicronemia syndrome. Promising TG lowering therapies include DGAT1 inhibition and intestinal specific MTP inhibition.

VI. References

1. **Ohsfeldt RL, Gandhi SK, Smolen LJ, Jensen MM, Fox KM, Gold A, Hsia J** Cost effectiveness of rosuvastatin in patients at risk of cardiovascular disease based on findings from the JUPITER trial. J Med Econ 13:428-437
2. **Tsuyuki RT, Bungard TJ** 2001 Poor adherence with hypolipidemic drugs: a lost opportunity. Pharmacotherapy 21:576-582
3. **Joy TR, Hegele RA** 2009 Narrative review: statin-related myopathy. Ann Intern Med 150:858-868
4. **Golomb BA, McGraw JJ, Evans MA, Dimsdale JE** 2007 Physician response to patient reports of adverse drug effects: implications for patient-targeted adverse effect surveillance. Drug Saf 30:669-675
5. **Phillips PS, Haas RH, Bannykh S, Hathaway S, Gray NL, Kimura BJ, Vladutiu GD, England JD** 2002 Statin-associated myopathy with normal creatine kinase levels. Ann Intern Med 137:581-585
6. **Pasternak RC, Smith SC, Jr., Bairey-Merz CN, Grundy SM, Cleeman JI, Lenfant C** 2002 ACC/AHA/NHLBI Clinical Advisory on the Use and Safety of Statins. Circulation 106:1024-1028
7. **McKenney JM, Davidson MH, Jacobson TA, Guyton JR** 2006 Final conclusions and recommendations of the National Lipid Association Statin Safety Assessment Task Force. Am J Cardiol 97:89C-94C
8. **Yamamoto A, Sudo H, Endo A** 1980 Therapeutic effects of ML-236B in primary hypercholesterolemia. Atherosclerosis 35:259-266
9. **Endo A** 1992 The discovery and development of HMG-CoA reductase inhibitors. J Lipid Res 33:1569-1582
10. **East C, Alivizatos PA, Grundy SM, Jones PH, Farmer JA** 1988 Rhabdomyolysis in patients receiving lovastatin after cardiac transplantation. N Engl J Med 318:47-48
11. **Norman DJ, Illingworth DR, Munson J, Hosenpud J** 1988 Myolysis and acute renal failure in a heart-transplant recipient receiving lovastatin. N Engl J Med 318:46-47
12. **Hansen KE, Hildebrand JP, Ferguson EE, Stein JH** 2005 Outcomes in 45 patients with statin-associated myopathy. Arch Intern Med 165:2671-2676
13. **Ghatak A, Faheem O, Thompson PD** 2010 The genetics of statin-induced myopathy. Atherosclerosis 210:337-343
14. **Vandenberg BF, Robinson J** Management of the patient with statin intolerance. Curr Atheroscler Rep 12:48-57
15. **Kashani A, Phillips CO, Foody JM, Wang Y, Mangalmurti S, Ko DT, Krumholz HM** 2006 Risks associated with statin therapy: a systematic overview of randomized clinical trials. Circulation 114:2788-2797
16. **Bruckert E, Hayem G, Dejager S, Yau C, Begaud B** 2005 Mild to moderate muscular symptoms with high-dosage statin therapy in hyperlipidemic patients--the PRIMO study. Cardiovasc Drugs Ther 19:403-414
17. **Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto AM, Jr., Kastelein JJ, Koenig W, Libby P, Lorenzatti AJ, MacFadyen JG, Nordestgaard BG, Shepherd J, Willerson JT, Glynn RJ** 2008 Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. N Engl J Med 359:2195-2207
18. **Cohen JD, Brinton EA, Ito MK, Jacobson TA** Understanding Statin Use in America and Gaps in Patient Education (USAGE): an internet-based survey of 10,138 current and former statin users. J Clin Lipidol 6:208-215
19. **Parker BA, Capizzi JA, Grimaldi AS, Clarkson PM, Cole SM, Keadle J, Chipkin S, Pescatello LS, Simpson K, White CM, Thompson PD** 2012 The Effect of Statins on Skeletal Muscle Function. Circulation
20. **Stein EA** 2009 Other therapies for reducing low-density lipoprotein cholesterol: medications in development. Endocrinol Metab Clin North Am 38:99-119
21. **Thompson PD, Zmuda JM, Domalik LJ, Zimet RJ, Staggers J, Guyton JR** 1997 Lovastatin increases exercise-induced skeletal muscle injury. Metabolism 46:1206-1210
22. **Schalke BB, Schmidt B, Toyka K, Hartung HP** 1992 Pravastatin-associated inflammatory myopathy. N Engl J Med 327:649-650

23. **McKelvie PA, Dennett X** 2002 Myopathy Associated With HMG-CoA Reductase Inhibitors (Statins): A Series of 10 Patients and Review of the Literature. J Clin Neuromuscul Dis 3:143-148
24. **Mohaupt MG, Karas RH, Babiychuk EB, Sanchez-Freire V, Monastyrskaya K, Iyer L, Hoppeler H, Breil F, Draeger A** 2009 Association between statin-associated myopathy and skeletal muscle damage. CMAJ 181:E11-18
25. **Draeger A, Monastyrskaya K, Mohaupt M, Hoppeler H, Savolainen H, Allemann C, Babiychuk EB** 2006 Statin therapy induces ultrastructural damage in skeletal muscle in patients without myalgia. J Pathol 210:94-102
26. **Mancini GB, Baker S, Bergeron J, Fitchett D, Frohlich J, Genest J, Gupta M, Hegele RA, Ng D, Pope J** 2011 Diagnosis, prevention, and management of statin adverse effects and intolerance: proceedings of a Canadian Working Group Consensus Conference. Can J Cardiol 27:635-662
27. **Ghirlanda G, Oradei A, Manto A, Lippa S, Uccioli L, Caputo S, Greco AV, Littarru GP** 1993 Evidence of plasma CoQ10-lowering effect by HMG-CoA reductase inhibitors: a double-blind, placebo-controlled study. J Clin Pharmacol 33:226-229
28. **Rundek T, Naini A, Sacco R, Coates K, DiMauro S** 2004 Atorvastatin decreases the coenzyme Q10 level in the blood of patients at risk for cardiovascular disease and stroke. Archives of neurology 61:889-892
29. **Paiva H, Thelen KM, Van Coster R, Smet J, De Paepe B, Mattila KM, Laakso J, Lehtimaki T, von Bergmann K, Lutjohann D, Laaksonen R** 2005 High-dose statins and skeletal muscle metabolism in humans: a randomized, controlled trial. Clin Pharmacol Ther 78:60-68
30. **Bleske BE, Willis RA, Anthony M, Casselberry N, Datwani M, Uhley VE, Secontine SG, Shea MJ** 2001 The effect of pravastatin and atorvastatin on coenzyme Q10. Am Heart J 142:E2
31. **Caso G, Kelly P, McNurlan MA, Lawson WE** 2007 Effect of coenzyme q10 on myopathic symptoms in patients treated with statins. Am J Cardiol 99:1409-1412
32. **Young JM, Florkowski CM, Molyneux SL, McEwan RG, Frampton CM, George PM, Scott RS** 2007 Effect of coenzyme Q(10) supplementation on simvastatin-induced myalgia. Am J Cardiol 100:1400-1403
33. **Bookstaver DA, Burkhalter NA, Hatzigeorgiou C** 2012 Effect of coenzyme Q10 supplementation on statin-induced myalgias. Am J Cardiol 110:526-529
34. **Gupta A, Thompson PD** 2011 The relationship of vitamin D deficiency to statin myopathy. Atherosclerosis 215:23-29
35. **Williams D, Feely J** 2002 Pharmacokinetic-pharmacodynamic drug interactions with HMG-CoA reductase inhibitors. Clin Pharmacokinet 41:343-370
36. **Kalliokoski A, Niemi M** 2009 Impact of OATP transporters on pharmacokinetics. British journal of pharmacology 158:693-705
37. **Niemi M, Pasanen MK, Neuvonen PJ** 2011 Organic anion transporting polypeptide 1B1: a genetically polymorphic transporter of major importance for hepatic drug uptake. Pharmacol Rev 63:157-181
38. **Link E, Parish S, Armitage J, Bowman L, Heath S, Matsuda F, Gut I, Lathrop M, Collins R** 2008 SLCO1B1 variants and statin-induced myopathy--a genomewide study. N Engl J Med 359:789-799
39. **Neuvonen PJ, Backman JT, Niemi M** 2008 Pharmacokinetic comparison of the potential over-the-counter statins simvastatin, lovastatin, fluvastatin and pravastatin. Clin Pharmacokinet 47:463-474
40. **Niemi M** 2010 Transporter pharmacogenetics and statin toxicity. Clin Pharmacol Ther 87:130-133
41. **Knauer MJ, Urquhart BL, Meyer zu Schwabedissen HE, Schwarz UI, Lemke CJ, Leake BF, Kim RB, Tirona RG** Human skeletal muscle drug transporters determine local exposure and toxicity of statins. Circ Res 106:297-306
42. **Cohen J, Pertsemlidis A, Kotowski IK, Graham R, Garcia CK, Hobbs HH** 2005 Low LDL cholesterol in individuals of African descent resulting from frequent nonsense mutations in PCSK9. Nat Genet 37:161-165
43. **Cohen JC, Boerwinkle E, Mosley TH, Jr., Hobbs HH** 2006 Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. N Engl J Med 354:1264-1272
44. **Sullivan D, Olsson AG, Scott R, Kim JB, Xue A, Gebski V, Wasserman SM, Stein EA** 2012 Effect of a Monoclonal Antibody to PCSK9 on Low-Density Lipoprotein Cholesterol Levels in Statin-Intolerant Patients: The GAUSS Randomized Trial. JAMA:1-10
45. **Garg A, Simha V** 2007 Update on dyslipidemia. J Clin Endocrinol Metab 92:1581-1589
46. **Cannon CP, Braunwald E, McCabe CH, Rader DJ, Rouleau JL, Belder R, Joyal SV, Hill KA, Pfeffer MA, Skene AM** 2004 Intensive versus moderate lipid lowering with statins after acute coronary syndromes. N Engl J Med 350:1495-1504

47. **LaRosa JC, Grundy SM, Waters DD, Shear C, Barter P, Fruchart JC, Gotto AM, Greten H, Kastelein JJ, Shepherd J, Wenger NK** 2005 Intensive lipid lowering with atorvastatin in patients with stable coronary disease. N Engl J Med 352:1425-1435
48. **Pedersen TR, Faergeman O, Kastelein JJ, Olsson AG, Tikkanen MJ, Holme I, Larsen ML, Bendiksen FS, Lindahl C, Szarek M, Tsai J** 2005 High-dose atorvastatin vs usual-dose simvastatin for secondary prevention after myocardial infarction: the IDEAL study: a randomized controlled trial. JAMA 294:2437-2445
49. **de Lemos JA, Blazing MA, Wiviott SD, Lewis EF, Fox KA, White HD, Rouleau JL, Pedersen TR, Gardner LH, Mukherjee R, Ramsey KE, Palmisano J, Bilheimer DW, Pfeffer MA, Califf RM, Braunwald E** 2004 Early intensive vs a delayed conservative simvastatin strategy in patients with acute coronary syndromes: phase Z of the A to Z trial. JAMA 292:1307-1316
50. **Khachadurian AK, Uthman SM** 1973 Experiences with the homozygous cases of familial hypercholesterolemia. A report of 52 patients. Nutrition and metabolism 15:132-140
51. **Goldstein JL, Brown MS** 1974 Binding and degradation of low density lipoproteins by cultured human fibroblasts. Comparison of cells from a normal subject and from a patient with homozygous familial hypercholesterolemia. J Biol Chem 249:5153-5162
52. **Leigh SE, Foster AH, Whittall RA, Hubbart CS, Humphries SE** 2008 Update and analysis of the University College London low density lipoprotein receptor familial hypercholesterolemia database. Ann Hum Genet 72:485-498
53. **Rader DJ, Cohen J, Hobbs HH** 2003 Monogenic hypercholesterolemia: new insights in pathogenesis and treatment. J Clin Invest 111:1795-1803
54. **Raal FJ, Pilcher GJ, Illingworth DR, Pappu AS, Stein EA, Laskarzewski P, Mitchel YB, Melino MR** 1997 Expanded-dose simvastatin is effective in homozygous familial hypercholesterolaemia. Atherosclerosis 135:249-256
55. **Huijgen R, Kindt I, Verhoeven SB, Sijbrands EJ, Vissers MN, Kastelein JJ, Hutten BA** Two years after molecular diagnosis of familial hypercholesterolemia: majority on cholesterol-lowering treatment but a minority reaches treatment goal. PLoS One 5:e9220
56. **Leren TP, Finborud TH, Manshaus TE, Ose L, Berge KE** 2008 Diagnosis of familial hypercholesterolemia in general practice using clinical diagnostic criteria or genetic testing as part of cascade genetic screening. Community Genet 11:26-35
57. **Hadfield SG, Horara S, Starr BJ, Yazdgerdi S, Bhatnagar D, Cramb R, Egan S, Everdell R, Ferns G, Jones A, Marenah CB, Marples J, Prinsloo P, Sneyd A, Stewart MF, Sandle L, Wang T, Watson MS, Humphries SE** 2008 Are patients with familial hypercholesterolaemia well managed in lipid clinics? An audit of eleven clinics from the Department of Health Familial Hypercholesterolaemia Cascade Testing project. Ann Clin Biochem 45:199-205
58. **Thompson GR, Lowenthal R, Myant NB** 1975 Plasma exchange in the management of homozygous familial hypercholesterolaemia. Lancet 1:1208-1211
59. **Thompson GR, Miller JP, Breslow JL** 1985 Improved survival of patients with homozygous familial hypercholesterolaemia treated with plasma exchange. Br Med J (Clin Res Ed) 291:1671-1673
60. **Kitano Y, Thompson GR** 1997 The familial hypercholesterolemia regression study: a randomized comparison of therapeutic reduction of both low-density lipoprotein and lipoprotein(a) versus low-density lipoprotein alone. Therapeutic apheresis : official journal of the International Society for Apheresis and the Japanese Society for Apheresis 1:187-190
61. **Kroon AA, Aengevaeren WR, van der Werf T, Uijen GJ, Reiber JH, Bruschke AV, Stalenhoef AF** 1996 LDL-Apheresis Atherosclerosis Regression Study (LAARS). Effect of aggressive versus conventional lipid lowering treatment on coronary atherosclerosis. Circulation 93:1826-1835
62. **Stein EA, Mellis S, Yancopoulos GD, Stahl N, Logan D, Smith WB, Lisbon E, Gutierrez M, Webb C, Wu R, Du Y, Kranz T, Gasparino E, Swergold GD** 2012 Effect of a monoclonal antibody to PCSK9 on LDL cholesterol. N Engl J Med 366:1108-1118
63. **Stein EA, Gipe D, Bergeron J, Gaudet D, Weiss R, Dufour R, Wu R, Pordy R** 2012 Effect of a monoclonal antibody to PCSK9, REGN727/SAR236553, to reduce low-density lipoprotein cholesterol in patients with heterozygous familial hypercholesterolaemia on stable statin dose with or without ezetimibe therapy: a phase 2 randomised controlled trial. Lancet 380:29-36

64. **Raal F, Scott R, Somaratne R, Bridges I, Li G, Wasserman SM, Stein EA** 2012 Low-Density Lipoprotein Cholesterol-Lowering Effects of AMG 145, a Monoclonal Antibody to Proprotein Convertase Subtilisin/Kexin Type 9 Serine Protease in Patients With Heterozygous Familial Hypercholesterolemia: The Reduction of LDL-C With PCSK9 Inhibition in Heterozygous Familial Hypercholesterolemia Disorder (RUTHERFORD) Randomized Trial. Circulation 126:2408-2417
65. **Puddu PE** [The JUPITER study: an orbit still very far from the Mediterranean countries]. G Ital Cardiol (Rome) 11:708-709
66. **Cuchel M, Bloedon LT, Szapary PO, Kolansky DM, Wolfe ML, Sarkis A, Millar JS, Ikewaki K, Siegelman ES, Gregg RE, Rader DJ** 2007 Inhibition of microsomal triglyceride transfer protein in familial hypercholesterolemia. N Engl J Med 356:148-156
67. **Raal FJ** 2012 Lomitapide for homozygous familial hypercholesterolaemia. Lancet
68. **Langlois S, Deeb S, Brunzell JD, Kastelein JJ, Hayden MR** 1989 A major insertion accounts for a significant proportion of mutations underlying human lipoprotein lipase deficiency. Proc Natl Acad Sci U S A 86:948-952
69. **Deep SS, Brunzell JD** 2006 Chapter 117: Familial Lipoprotein Lipase Deficiency, Apo C-II Deficiency, and Hepatic Lipase Deficiency. In: Metabolic and Molecular Bases of Inherited Disease: The McGraw-Hill Companies
70. **Priore Oliva C, Carubbi F, Schaap FG, Bertolini S, Calandra S** 2008 Hypertriglyceridaemia and low plasma HDL in a patient with apolipoprotein A-V deficiency due to a novel mutation in the APOA5 gene. J Intern Med 263:450-458
71. **Marcais C, Verges B, Charriere S, Pruneta V, Merlin M, Billon S, Perrot L, Drai J, Sassolas A, Pennacchio LA, Fruchart-Najib J, Fruchart JC, Durlach V, Moulin P** 2005 Apoa5 Q139X truncation predisposes to late-onset hyperchylomicronemia due to lipoprotein lipase impairment. J Clin Invest 115:2862-2869
72. **Peterfy M, Ben-Zeev O, Mao HZ, Weissglas-Volkov D, Aouizerat BE, Pullinger CR, Frost PH, Kane JP, Malloy MJ, Reue K, Pajukanta P, Doolittle MH** 2007 Mutations in LMF1 cause combined lipase deficiency and severe hypertriglyceridemia. Nat Genet 39:1483-1487
73. **Beigneux AP, Franssen R, Bensadoun A, Gin P, Melford K, Peter J, Walzem RL, Weinstein MM, Davies BS, Kuivenhoven JA, Kastelein JJ, Fong LG, Dallinga-Thie GM, Young SG** 2009 Chylomicronemia With a Mutant GPIHBP1 (Q115P) That Cannot Bind Lipoprotein Lipase. Arterioscler Thromb Vasc Biol
74. **Gaudet D, de Wal J, Tremblay K, Dery S, van Deventer S, Freidig A, Brisson D, Methot J** 2010 Review of the clinical development of alipogene tiparvovec gene therapy for lipoprotein lipase deficiency. Atheroscler Suppl 11:55-60
75. **Chen HC, Farese RV, Jr.** 2005 Inhibition of triglyceride synthesis as a treatment strategy for obesity: lessons from DGAT1-deficient mice. Arterioscler Thromb Vasc Biol 25:482-486
76. **Haas JT, Winter HS, Lim E, Kirby A, Blumenstiel B, Defelice M, Gabriel S, Jalas C, Branski D, Grueter CA, Toporovski MS, Walther TC, Daly MJ, Farese RV, Jr.** 2012 DGAT1 mutation is linked to a congenital diarrheal disorder. J Clin Invest 122:4680-4684